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Supplementary Materials for

The Balance of WNT and FGF Signaling Influences Mesenchymal Stem Cell Fate During Skeletal Development

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Published 25 May 2010, *Sci. Signal.* **3**, ra40 (2010)
DOI: 10.1126/scisignal.2000727

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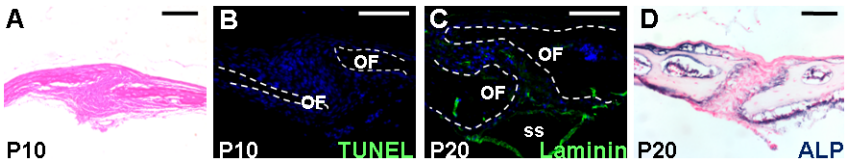

Figure S1. Endochondral ossification is not involved in normal development of the SAG suture. (**A**) Alcian blue (AB) staining and (**B**) TUNEL staining, performed on P10 animal skulls, and (**C**) immunostaining for laminin and (**D**) alkaline phosphatase (ALP) staining, performed on P20 animal skulls, show no sign of bone mineralization mediated by endochondral ossification in wild-type animals. OF, osteogenic front; SS, sagittal sinus. Scale bars, 100 μm.

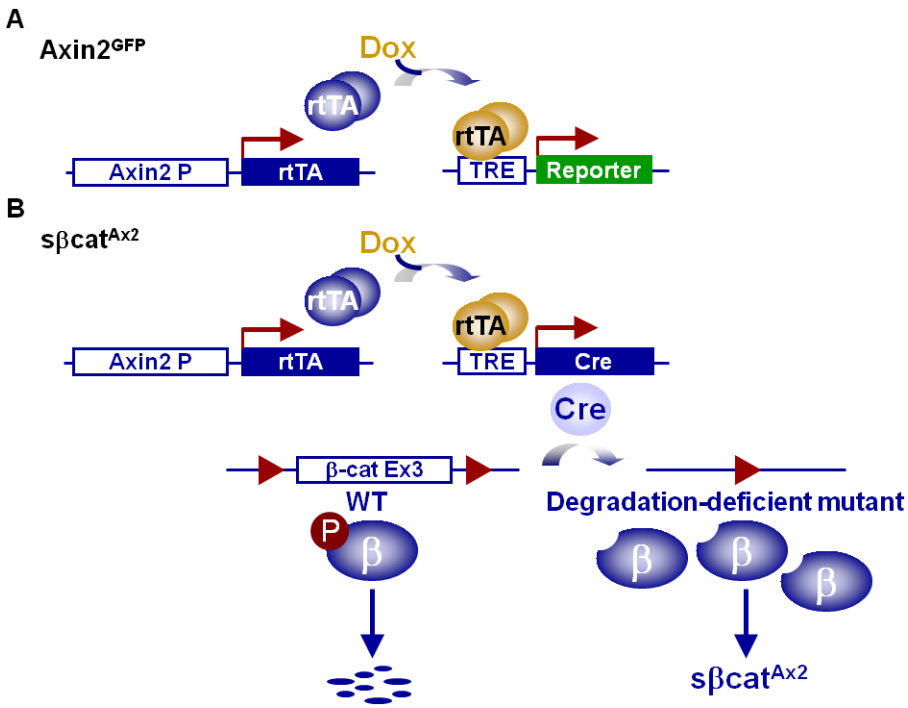

Figure S2. Diagrams illustrating the generation of transgenic mice used to monitor the expression of *Axin2* or produce a stabilized form of β-catenin. (**A**) In the Axin2^{GFP} mice, production of the transcription factor rtTA, a fusion protein containing the reverse tet repressor and viral VP16 fragments, is controlled by the *Axin2* promoter, and GFP production is controlled by the TRE, which is activated by rtTA. In the presence of doxycycline and in cells in which *Axin2* is expressed, rtTA is produced and stimulates the production of GFP, allowing *Axin2* expression to be monitored as GFP fluorescence. (**B**) In the sβcat^{Ax2} mouse, cells in which *Axin2* is expressed and in the presence of doxycycline, they produce a constitutively stabilized form of β-catenin. The sβcat^{Ax2} mutants are useful for studying the stimulatory effects of β-catenin signaling in the *Axin2*-expressing cells.

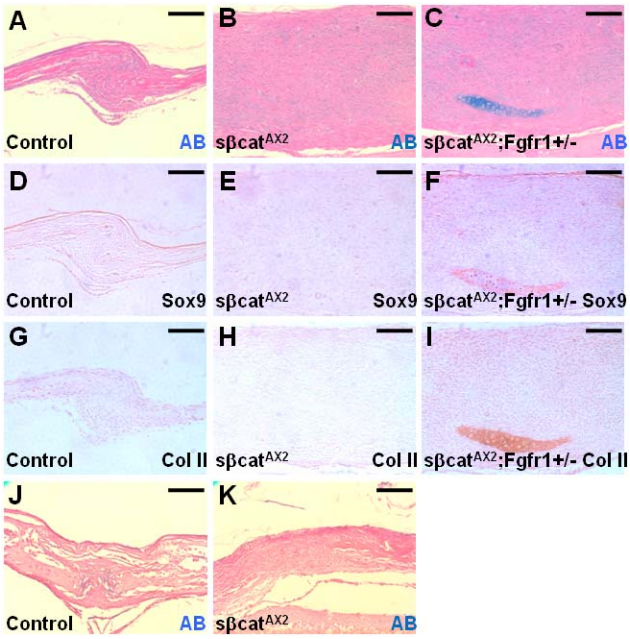

Figure S3. Increased β-catenin signaling combined with reduced FGFR1 signaling induces ectopic chondrogenesis in the SAG suture. Sections of the control (TRE-Cre; β-cateninΔEx3Fx/+) (A, D, G, J), sβcat^{Ax2} (Axin2-rtTA; TRE-Cre; β-cateninΔEx3Fx/+) (B, E, H, K) and sβcat^{Ax2}; Fgfr1+/- (Axin2-rtTA; TRE-Cre; β-cateninΔEx3Fx/+; Fgfr1+/-) (C, F, I) P7 SAG (A-I) and PF (J, K) sutures were analyzed by AB staining with eosin counterstaining (A-C, J, K), and immunostaining of Sox9 (D-F) and Col II (G-I). Cells positive for AB, Sox9, or Col II were only detected in the SAG sutures of sβcat^{Ax2}; Fgfr1+/- (n=3) but not those of control (n=3) or sβcat^{Ax2} (n=5). Scale bars, 100 μm.